

### REMARKS

The Examiner requested that a substitute sequence listing be submitted that included the sequences set forth in claims 1, 2, 4, 5, 16, 17, 26, 28, 30, 39, 42, 44, and 46. Applicants have submitted a substitute Sequence Listing as required by 37 C.F.R. §1.825(a), under separate cover, to the U.S. Patent and Trademark Office, Mail Stop - Box Sequence, PO Box 2327, Arlington, VA 22202, so that the computer readable form of the sequence listing would not be damaged. The sequence listing was amended to incorporate the amino acid motif recited in claim 5. In addition, Applicants submitted a paper copy of the substitute sequence listing as required under 37 C.F.R. §1.825(b) to the same address.

Claims 2, 5, 17, 39, 42, 44, and 46 have been amended to include the appropriate sequence identifier. Applicants note that the His-Xaa-Xaa-Xaa-His amino acid motif recited in claims 1, 4, 16, 26, 28, and 30, and at page 10, does not require a sequence identifier as the motif has fewer than four specifically defined amino acids. See, 37 C.F.R. §1.821(a).

### Marked-Up Copy of Amendment

The Examiner requested that a new marked up copy of the amendment filed January 24, 2002, be submitted that reflects all of the changes that were made. Submitted herewith is a new marked up copy of the amendment of January 24, 2002, which indicates all the changes that were made in the amendment. Applicants note that the changes to the paragraph beginning at page 44, line 19 and to the paragraph beginning at page 49, line 5, were made in the preliminary amendment of January 29, 2001. Thus, those paragraphs have been excluded from the "version with markings to show changes made" as there are no changes to indicate.

### Drawings

The Examiner also requested that new drawings be submitted. Formal drawings are submitted herewith.

Applicant : Lorin R. DeBonte, et al.  
Serial No. : 09/771,904  
Filed : January 29, 2001  
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Attorney's Docket No.: 07148-063003 / A15-505.35

CONCLUSION

Attached is a marked-up version of the changes being made by the current amendment.

Applicants ask that claims 1-3, 7-9, 11, 16-19, and 35-40 be examined. Please apply any other charges or credits to Deposit Account No. 06-1050.

Respectfully submitted,

Date: \_\_\_\_\_

2/7/03



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**Version with markings to show changes made**

**In the specification:**

Paragraph beginning at page 12, line 21 has been amended as follows:

Another region suitable for a mutation in a delta-12 desaturase sequence contains the motif KYLNNP (SEQ ID NO:64) at nucleotides corresponding to amino acids 170 to 175 of the Brassica desaturase sequence. An illustrative example of a mutation [is] in this region is a Leu to His substitution, resulting in the amino acid sequence (Table 4) KYHNNP (SEQ ID NO:53, compare wild-type Fad2-F amino acids 170-175 of SEQ ID NO:14 to mutant Fad2-F amino acids 170-175 of SEQ ID NO:16). A similar mutation in other Fad-2 amino acid sequences is contemplated to result in a non-functional gene product. (Compare SEQ ID NO:6 to SEQ ID NO:8).

**In the claims:**

Claims 2, 5, 17, 39, 42, and 44-46 have been amended as follows:

2. (Amended) The nucleic acid fragment of claim 1, wherein said at least one mutation comprises a mutation in a region of said desaturase gene encoding a His-Glu-Cys-Gly-His (SEQ ID NO:60) amino acid motif.

5. (Amended) The nucleic acid fragment of claim 4, wherein said at least one mutation comprises a mutation in a region of said desaturase gene encoding a His-Asp-Cys-Gly-His (SEQ ID NO:70) amino acid motif.

17. (Amended) The plant of claim 16, wherein said motif comprises the sequence His-Glu-Cys-Gly-His (SEQ ID NO:60).

39. (Amended) The plant of claim 37, wherein said motif comprises the sequence His-Glu-Cys-Gly-His (SEQ ID NO:60).

42. (Amended) The method of claim 41, wherein said identifying step comprises identifying a mutation in a His-Glu-Cys-Gly-His (SEQ ID NO:60) amino acid motif.

44. (Twice amended) The method of claim 28, wherein said identifying step comprises identifying a mutation in a His-Glu-Cys-Gly-His (SEQ ID NO:60) amino acid motif.

45. (Twice amended) The method of claim 28, wherein said producing step h) comprises producing seeds yielding an oil having an [ $\alpha$ -linolenic]  $\alpha$ -linolenic acid content from about 0.5% to about 10%.

46. (Amended) The method of claim 30, wherein said identifying step b) comprises identifying a mutation in a His-Glu-Cys-Gly-His (SEQ ID NO:60) amino acid motif.



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant : Lorin R. DeBonte, et al.

Art Unit : 1638

Serial No. : 09/771,904

Examiner : Elizabeth F. McElwain

Filed : January 29, 2001

Title : FATTY ACID DESATURASES AND MUTANT SEQUENCES THEREOF

Commissioner for Patents  
Washington, DC 20231

**Version with Markings to Show Changes**  
**Made in January 24, 2002, Amendment**

**In the specification:**

Paragraph beginning at page 6, line 4 has been amended as follows:

Figure 2 shows the nucleotide sequences for a *Brassica* Fad2-D wild type gene (Fad2-D wt; SEQ ID NO:9), IMC129 mutant gene (Fad2-D GA316 IMC129; SEQ ID NO:11), Fad2-F wild type gene (Fad2-F wt; SEQ ID NO:13), Q508 mutant gene (Fad2-F TA515 Q508; SEQ ID NO:15) and Q4275 mutant gene (Fad2-F GA908 Q4275; SEQ ID NO:17).

Paragraph beginning at page 6, line 9 has been amended as follows:

Figure 3 shows the deduced amino acid sequences (SEQ ID NOS:10, 12, 14, 16, and 18) for the polynucleotides of Figure 2.

Paragraph beginning at page 10, line 29 has been amended as follows:

Preferred mutations are in a region of the nucleic acid encoding an amino acid sequence motif that is conserved among delta-12 fatty acid desaturases or delta-15 fatty acid desaturases, such as a His-Xaa-Xaa-Xaa-His motif (Tables 1-3). An example of a suitable region has a conserved HECGH motif (SEQ ID NO:60) that is found, for example, in nucleotides corresponding to amino acids 105 to 109 of the *Arabidopsis* and *Brassica* delta-12 desaturase sequences, in nucleotides corresponding to amino acids 101 to 105 of the soybean delta-12 desaturase sequence and in nucleotides corresponding to amino acids 111 to 115 of the maize delta-12 desaturase sequence. See e.g., WO 94/115116; Okuley et al., Plant Cell 6:147-158

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(1994). The one letter amino acid designations used herein are described in Alberts, B. et al., Molecular Biology of the Cell, 3rd edition, Garland Publishing, New York, 1994. Amino acids flanking this motif are also highly conserved among delta-12 and delta-15 desaturases and are also suitable candidates for mutations in fragments of the invention.

Paragraph beginning at page 11, line 15 has been amended as follows:

An illustrative embodiment of a mutation in a nucleic acid fragment of the invention is a Glu to Lys substitution in the HECGH (SEQ ID NO:60) motif of a *Brassica* microsomal delta-12 desaturase sequence, either the D form or the F form. This mutation results in the sequence HECGH (SEQ ID NO:60) being changed to HKCGH (SEQ ID NO:58) as seen by comparing amino acids 105-109 of SEQ ID NO:10 (wild-type D form) to amino acids 105-109 of SEQ ID NO:12 (mutant D form). A similar mutation in other Fad-2 sequences is contemplated to result in a non-functional gene product. (Compare SEQ ID NO:2 to SEQ ID NO:4).

Paragraph beginning at page 11, line 32 has been amended as follows:

Among the types of mutations in an HECGH (SEQ ID NO:60) motif that render the resulting gene product non-functional are non-conservative substitutions. An illustrative example of a non-conservative substitution is substitution of a glycine residue for either the first or second histidine. Such a substitution replaces a charged residue (histidine) with a non-polar residue (glycine). Another type of mutation that renders the resulting gene product non-functional is an insertion mutation, e.g., insertion of a glycine between the cysteine and glutamic acid residues in the HECGH (SEQ ID NO:60) motif.

Paragraph beginning at page 12, line 9 has been amended as follows:

Other regions having suitable conserved amino acid motifs include the HRRHH motif (SEQ ID NO:61) shown in Table 2, the HRTHH motif (SEQ ID NO:62) shown in Table 6 and the HVAHH motif (SEQ ID NO:63) shown in Table 3. See, e.g., WO 94/115116; Hitz, W. et al.,

Plant Physiol., 105:635-641 (1994); Okuley, J., et al., supra; and Yadav, N. et al., supra. An illustrative example of a mutation in the region shown in Table 3 is a mutation at nucleotides corresponding to the codon for glycine (amino acid 303 of *B. napus*): A non-conservative Gly to Glu substitution results in the amino acid sequence DRDYGILNKV (SEQ ID NO:47, amino acids 299-308 of SEQ ID NO:14) being changed to sequence DRDYEILNKV (SEQ ID NO:50, amino acids 299-308 of SEQ ID NO:18) (compare wild-type F form SEQ ID NO:14 to mutant Q4275 SEQ ID NO:18, Fig. 3).

Paragraph beginning at page 12, line 21 has been amended as follows:

Another region suitable for a mutation in a delta-12 desaturase sequence contains the motif KYLNNP (SEQ ID NO:64) at nucleotides corresponding to amino acids [171] 170 to 175 of the *Brassica* desaturase sequence. An illustrative example of a mutation in this region is a Leu to His substitution, resulting in the amino acid sequence (Table 4) KYHNNP (SEQ ID NO:53, compare wild-type Fad2-F amino acids 170-175 of SEQ ID NO:14 to mutant Fad2-F amino acids 170-175 of SEQ ID NO:16). A similar mutation in other Fad-2 amino acid sequences is contemplated to result in a non-functional gene product. (Compare SEQ ID NO:6 to SEQ ID NO:8).

Table 1 on page 13, has been amended as follows:

**TABLE 1**  
Alignment of Amino Acid Sequences from Microsomal  
Delta-12 Fatty Acid Desaturases

| Species                              | Position             | Amino Acid Sequence                                      |
|--------------------------------------|----------------------|--|
| <i>Arabidopsis thaliana</i>          | 100-129              | IWVIAHECGH HAFSDYQWLD DTVGLIFHSF ( <u>SEQ ID NO:27</u> ) |
| <i>Glycine max</i>                   | 96-125               | VWVIAHECGH HAFSKYQWVD DVVGLTLHST ( <u>SEQ ID NO:28</u> ) |
| <i>Zea mays</i>                      | 106-135              | VWVIAHECGH HAFSDYSLLD DVVGLVLHSS ( <u>SEQ ID NO:29</u> ) |
| <i>Ricinus communis</i> <sup>a</sup> | 1- 29                | WVMAHDCGH HAFSDYQLLD DVVGLILHSC ( <u>SEQ ID NO:30</u> )  |
| <i>Brassica napus D</i>              | 100-128 <sup>b</sup> | VWVIAHECGH HAFSDYQWLD DTVGLIFHS ( <u>SEQ ID NO:65</u> )  |
| <i>Brassica napus F</i>              | 100-128 <sup>c</sup> | VWVIAHECGH HAFSDYQWLD DTVGLIFHS ( <u>SEQ ID NO:65</u> )  |

<sup>a</sup> from plasmid pRF2-1C, <sup>b</sup>positions 100-128 of SEQ ID NO:10; <sup>c</sup>positions 100-128 of SEQ ID NO:14

Table 2 on page 13, has been amended as follows:

**TABLE 2**

Alignment of Amino Acid Sequences from Microsomal  
Delta-12 Fatty Acid Desaturases

| Species                              | Position             | Amino Acid Sequence                            |
|--------------------------------------|----------------------|--|
| <i>Arabidopsis thaliana</i>          | 130-158              | LLVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:31) |
| <i>Glycine max</i>                   | 126-154              | LLVPYFSWKI SHRRHHSNTG SLDRDEVFV (SEQ ID NO:32) |
| <i>Zea mays</i>                      | 136-164              | LMVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:33) |
| <i>Ricinus communis</i> <sup>a</sup> | 30- 58               | LLVPYFSWKH SHRRHHSNTG SLERDEVFV (SEQ ID NO:34) |
| <i>Brassica napus D</i>              | 130-158 <sup>b</sup> | LLVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:31) |
| <i>Brassica napus F</i>              | 130-158 <sup>c</sup> | LLVPYFSWKY SHRRHHSNTG SLERDEVFV (SEQ ID NO:31) |

<sup>a</sup> from plasmid pRF2-1C; <sup>b</sup>positions 130-158 of SEQ ID NO:10; <sup>c</sup>positions 130-158 of SEQ ID NO:14

Table 3 on page 13, has been amended as follows:

**TABLE 3**

Alignment of Amino Acid Sequences from Microsomal  
Delta-12 Fatty Acid Desaturases

| Species                              | Position             | Amino Acid Sequence                                    |
|--------------------------------------|----------------------|--|
| <i>Arabidopsis thaliana</i>          | 298-333              | DRDYGILNKV FHNITDTHVA HHLFSTMPHY NAMEAT (SEQ ID NO:35) |
| <i>Glycine max</i>                   | 294-329              | DRDYGILNKV FHHITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:36) |
| <i>Zea mays</i>                      | 305-340              | DRDYGILNRV FHNITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:37) |
| <i>Ricinus communis</i> <sup>a</sup> | 198-224              | DRDYGILNKV FHNITDTQVA HHLF TMP (SEQ ID NO:38)          |
| <i>Brassica napus D</i>              | 299-334 <sup>b</sup> | DRDYGILNKV FHNITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:66) |
| <i>Brassica napus F</i>              | 299-334 <sup>c</sup> | DRDYGILNKV FHNITDTHVA HHLFSTMPHY HAMEAT (SEQ ID NO:66) |

<sup>a</sup> from plasmid pRF2-1C; <sup>b</sup>positions 299-334 of SEQ ID NO:10; <sup>c</sup>positions 299-334 of SEQ ID NO:14



Table 4 on page 14, has been amended as follows:

**TABLE 4**

Alignment of Conserved Amino Acids from Microsomal  
Delta-12 Fatty Acid Desaturases

| Species                              | Position             | Amino Acid Sequence              |
|--------------------------------------|----------------------|----------------------------------|
| <i>Arabidopsis thaliana</i>          | 165-180              | IKWYGKYLNN PLGRIM (SEQ ID NO:39) |
| <i>Glycine max</i>                   | 161-176              | VAWFSLYLNN PLGRAV (SEQ ID NO:40) |
| <i>Zea mays</i>                      | 172-187              | PWYTPYVYNN PVGRVV (SEQ ID NO:41) |
| <i>Ricinus communis</i> <sup>a</sup> | 65- 80               | IRWYSKYLNN PPGRIM (SEQ ID NO:42) |
| <i>Brassica napus</i> D              | 165-180 <sup>b</sup> | IKWYGKYLNN PLGRTV (SEQ ID NO:67) |
| <i>Brassica napus</i> F              | 165-180 <sup>c</sup> | IKWYGKYLNN PLGRTV (SEQ ID NO:67) |

<sup>a</sup> from plasmid pRF2-1C; <sup>b</sup>positions 165-180 of SEQ ID NO:10; <sup>c</sup>positions 165-180 of SEQ ID NO:14

Table 5 on page 14, has been amended as follows:

**TABLE 5**

Alignment of Conserved Amino Acids from Plastid and Microsomal  
Delta-15 Fatty Acid Desaturases

| Species                                  | Position | Amino Acid Sequence                     |
|--|----------|---|
| <i>Arabidopsis thaliana</i> <sup>a</sup> | 156-177  | WALFVLGHD CGHGSFSNDP KLN (SEQ ID NO:43) |
| <i>Brassica napus</i> <sup>a</sup>       | 114-135  | WALFVLGHD CGHGSFSNDP RLN (SEQ ID NO:44) |
| <i>Glycine max</i> <sup>a</sup>          | 164-185  | WALFVLGHD CGHGSFSNNS KLN (SEQ ID NO:45) |
| <i>Arabidopsis thaliana</i>              | 94-115   | WAIFVLGHD CGHGSFSDIP LLN (SEQ ID NO:46) |
| <i>Brassica napus</i>                    | 87-109   | WALFVLGHD CGHGSFSNDP RLN (SEQ ID NO:44) |
| <i>Glycine max</i>                       | 93-114   | WALFVLGHD CGHGSFSDSP PLN (SEQ ID NO:48) |

<sup>a</sup> Plastid sequences

Table 6 on page 14, has been amended as follows:

**TABLE 6**

Alignment of Conserved Amino Acids from Plastid and Microsomal  
 Delta-15 Fatty Acid Desaturases

| Species                         | Position | Amino Acid Sequence                                     |
|---------------------------------|----------|---|
| <i>A. thaliana</i> <sup>a</sup> | 188-216  | ILVPYHGWRI SHRTHHQNHG HVENDESWH ( <u>SEQ ID NO:49</u> ) |
| <i>B. napus</i> <sup>a</sup>    | 146-174  | ILVPYHGWRI SHRTHHQNHG HVENDESWH ( <u>SEQ ID NO:49</u> ) |
| <i>Glycine max</i> <sup>a</sup> | 196-224  | ILVPYHGWRI SHRTHHQNHG HAENDESWH ( <u>SEQ ID NO:51</u> ) |
| <i>A. thaliana</i>              | 126-154  | ILVPYHGWRI SHRTHHQNHG HVENDESWV ( <u>SEQ ID NO:52</u> ) |
| <i>Brassica napus</i>           | 117-145  | ILVPYHGWRI SHRTHHQNHG HVENDESWV ( <u>SEQ ID NO:52</u> ) |
| <i>Glycine max</i>              | 125-153  | ILVPYHGWRI SHRTHHQNHG HIEKDESWV ( <u>SEQ ID NO:54</u> ) |

<sup>a</sup> Plastid sequences

Paragraph beginning at page 17, line 1, has been amended as follows:

The seeds of several different fatty acid lines have been deposited with the American Type Culture Collection, 10801 University Blvd., Manassas, Virginia 20110-2209, and have the following accession numbers.

Table 20 on page 44, has been amended as follows:

**TABLE 20**

Alignment of Amino Acid Sequences  
 of Cloned Canola Membrane Bound-Desaturases

| Desaturase Gene       | Sequence <sup>a</sup>          | Position                       |
|-----------------------|--------------------------------|--------------------------------|
| Canola-fad2-D(mutant) | AHKCGH ( <u>SEQ ID NO:68</u> ) | 109-114 of <u>SEQ ID NO:12</u> |
| Canola-Fad2-D         | AHECGH ( <u>SEQ ID NO:69</u> ) | 109-114 of <u>SEQ ID NO:10</u> |
| Canola-Fad2-F         | AHECGH ( <u>SEQ ID NO:69</u> ) | 109-114 of <u>SEQ ID NO:14</u> |
| Canola-FadC           | GHDCAH ( <u>SEQ ID NO:55</u> ) | 170-175                        |
|                       |                                |                                |
| Canola-fad3 (mutant)  | GHKCGH ( <u>SEQ ID NO:56</u> ) | 94-99                          |

| Desaturase Gene | Sequence <sup>a</sup>          | Position |
|-----------------|--------------------------------|----------|
| Canola-Fad3     | GHDCGH ( <u>SEQ ID NO:57</u> ) | 94-99    |
| Canola-FadD     | GHDCGH ( <u>SEQ ID NO:57</u> ) | 125-130  |

(FadD = Plastid delta 15, Fad3 = Microsomal delta-15),  
(FadC = Plastid delta-12, Fad2 = Microsomal delta-12)

<sup>a</sup> One letter amino acid code; conservative substitutions are underlined; non-conservative substitutions are in bold.

Paragraph beginning at page 46, line 26 has been amended as follows:

The second plasmid, pIMC205, was prepared by inserting a mutated Fad3 gene in sense orientation into a disarmed Ti vector. The mutant sequence contained mutations at nucleotides 411 and 413 of the microsomal Fad3 gene described in WO93/11245, thus changing the sequence for codon 96 from GAC to AAG. The amino acid at codon 96 of the gene product was thereby changed from aspartic acid to lysine. See Table 20. A bean (*Phaseolus vulgaris*) phaseolin (7S seed storage protein) promoter fragment of 495 base pairs, starting with 5'-TGGTCTTTTGGT-3' (SEQ ID NO:59), was placed 5' to the mutant Fad3 gene and a phaseolin termination sequence was placed 3' to the mutant Fad3 gene. The phaseolin sequence is described in Doyle et al., (1986) J. Biol. Chem. 261:9228-9238) and Slightom et al., (1983) Proc. Natl. Acad. Sci. USA 80:1897-1901.

Paragraph beginning at page 49, line 5 has been amended as follows:

The Fad2-D gene was amplified once using Elongase<sup>®</sup> (Gibco-BRL). PCR primers were: 5'-CAUCAUCAUCAUCTTCTTCGTAGGGTTCATCG-3' (SEQ ID NO:23) and 5'-CUACUACUACUATCATAGAAGAGAAAGGTTTCAG-3' (SEQ ID NO:24) for the 5' and 3' ends of the gene, respectively.